

## CLAIMS

We claim:

- 5 1. A pest control agent comprising at least one expression vector, wherein the at least one expression vector directs transcription of at least one ribonucleic acid (RNA) that, when present within a cell, forms a double-stranded structure that inhibits expression of at least one gene expressed in the cell.
- 10 2. The agent of claim 1 wherein the RNA comprises two separate complementary strands.
- 15 3. The agent of claim 2 wherein the expression vector comprises a DNA segment flanked by two promoters, wherein the promoters are operably linked to the DNA segment, and wherein the promoters are oriented so as to direct transcription of both sense and antisense RNA transcripts from the flanked DNA segment.
- 20 4. The agent of claim 3 wherein the expression vector further comprises DNA sequences that direct termination of the RNA transcripts.
5. The agent of claim 3 or claim 4 wherein the expression vector further comprises at least one enhancer element operably linked to at least one of the promoters.
- 25 6. The agent of claim 1 wherein the RNA comprises one strand that is self-complementary.

7. The agent of claim 1 wherein at least a portion of the ribonucleic acid sequence is identical to at least a portion of the sequence of the at least one gene.

8. The agent of claim 1 wherein the cell forms part of a tissue of a target pest organism.

9. The agent of claim 1 wherein the expression vector is a virus.

10. The agent of claim 9 wherein the virus is selected from the group consisting of: baculoviruses, entomopoxviruses, densoviruses, nodaviruses, nudiviruses, and shrimp viruses.

11. The agent of claim 10 wherein the virus is selected from the group consisting of: the *Autographa californica* multiple polyhedrosis virus, the *Orgyia pseudotsugata* MNPV, the *Lymantria dispar* MNPV, the *Helicoverpa zea* NPV, and the *Bombyx mori* NPV.

12. The agent of claim 3 wherein the promoters are viral promoters.

13. The agent of claim 3 wherein the promoters are baculovirus promoters.

14. The agent of claim 1 wherein at least one gene to be inhibited is an essential gene in a pest organism.

15. The agent of claim 1 wherein at least one gene to be inhibited is a gene involved

in development in a pest organism.

16. The agent of claim 1 wherein at least one gene to be inhibited is involved in neurotransmission in a pest organism.

17. The agent of claim 1 wherein at least one gene to be inhibited is expressed in the insect alimentary canal or Malpighian tubules.

18. The agent of claim 1 wherein the at least one gene to be inhibited is naturally found in an insect selected from the order *Lepidoptera*.

19. The agent of claim 1 wherein the at least one gene to be inhibited is naturally found in an insect selected from the list consisting of: the cotton bollworm (*Helicoverpa zea*), the cabbage looper (*Trichoplusia ni*), the alfalfa looper (*Autographa californica*), the tobacco hornworm (*Manduca sexta*), the tobacco budworm (*Heliothis virescens*), the fall armyworm (*Spodoptera frugiperda*), the European corn borer (*Ostrinia nubilalis*), the eastern spruce budworm (*Choristoneura fumiferana*), the western spruce budworm (*C. occidentalis*), and the gypsy moth (*Lymantria dispar*).

20. The agent of claim 1 wherein at least one gene to be inhibited is selected from the group consisting of *Drosophila hunchback*, *Drosophila melanogaster white*, *Drosophila melanogaster para*, *Drosophila melanogaster Hyperkinetic*, *Drosophila melanogaster fasciclin II*, *Drosophila melanogaster ecdysone receptor*, *Drosophila melanogaster vha55*, *Drosophila melanogaster vha26*, and *Manduca sexta* homologs of the aforementioned genes.

21. The agent of claim 1, wherein the RNA is species specific in that it does not show more than approximately 80% homology with any known gene in any species other than the species in which the RNA sequence is naturally found.

5 22. The agent of claim 1, wherein the RNA is species specific in that it does not show more than approximately 80% homology with any known vertebrate gene.

23. A recombinant insect virus comprising:

10 a first promoter,

a second promoter,

a DNA segment whose sequence comprises at least one sequence at least 80%

identical to a portion of the sequence of at least one insect gene,

wherein the portion of the sequence of the at least one insect gene is at

15 least 50 nucleotides in length,

a first enhancer operably linked to the first promoter,

a second enhancer operably linked to the second promoter,

a first transcriptional terminator, wherein the first transcriptional terminator is

positioned so as to terminate transcription directed by the first promoter,

20 a second transcriptional terminator, wherein the second transcriptional

terminator is positioned so as to terminate transcription directed by the

second promoter,

wherein the two promoters are operably linked to the DNA segment, and

wherein the promoters are oriented so as to direct transcription of both

25 sense and antisense RNA transcripts from the DNA segment.

24. The recombinant insect virus of claim 23, wherein the at least one insect gene is selected from the list consisting of: genes that are essential in a pest organism, genes involved in neurotransmission in a pest organism, genes involved in development in a pest organism, and genes expressed in the insect alimentary canal or Malpighian tubules.

25. The recombinant insect virus of claim 23, wherein the DNA segment is species specific in that it does not show more than approximately 80% homology with any known gene in any species other than the species in which the gene is naturally found.

26. The recombinant insect virus of claim 25, wherein the DNA segment is species specific in that it does not show more than approximately 80% homology with any known vertebrate gene.

27. A pest control agent comprising at least one recombinant insect virus, wherein the at least one recombinant insect virus directs transcription of at least one ribonucleic acid (RNA) that, when present within a cell, forms a double-stranded structure that inhibits expression of at least one gene expressed in the cell.

28. A pest control agent comprising an occlusion body containing a plurality of recombinant insect viruses, wherein each of the recombinant insect viruses directs transcription of at least one ribonucleic acid (RNA) that, when present within a cell, forms a double-stranded structure that inhibits expression of at least one gene expressed in the cell.

29. The recombinant insect virus of claim 27, wherein the at least one insect gene is

selected from the list consisting of: genes that are essential in a pest organism, genes involved in neurotransmission in a pest organism, genes involved in development in a pest organism, and genes expressed in the insect alimentary canal or Malpighian tubules.

30. The recombinant insect virus of claim 27, wherein the at least one insect gene is a gene naturally found in an insect selected from the order *Lepidoptera*.

31. The recombinant insect virus of claim 27, wherein the at least one insect gene is a gene naturally found in an insect selected from the list consisting of: the cotton bollworm (*Helicoverpa zea*), the cabbage looper (*Trichoplusia ni*), the alfalfa looper (*Autographa californica*), the tobacco hornworm (*Manduca sexta*), the tobacco budworm (*Heliothis virescens*), the fall armyworm (*Spodoptera frugiperda*), the European corn borer (*Ostrinia nubilalis*), the eastern spruce budworm (*Choristoneura fumiferana*), the western spruce budworm (*C. occidentalis*), and the gypsy moth (*Lymantria dispar*).

32. The recombinant insect virus of any of claims 24, 25, 26, 27, 28, 29 or 30, wherein the recombinant insect virus is a baculovirus.

33. A recombinant baculovirus comprising:

a first promoter,

a second promoter,

a DNA segment whose sequence comprises at least one sequence at least 80% identical to a portion of the sequence of at least one insect gene,

wherein the portion of the sequence of the at least one insect gene is at least 50 nucleotides in length,

a first enhancer operably linked to the first promoter,

a second enhancer operably linked to the second promoter,

a first transcriptional terminator, wherein the first transcriptional terminator is positioned so as to terminate transcription directed by the first promoter,

a second transcriptional terminator, wherein the second transcriptional terminator is positioned so as to terminate transcription directed by the second promoter,

wherein the two promoters are operably linked to the DNA segment, and

wherein the promoters are oriented so as to direct transcription of both sense and antisense RNA transcripts from the DNA segment.

34. A plasmid comprising:

a first promoter,

a second promoter, and

a site for insertion of a DNA segment,

wherein the two promoters are arranged in opposite orientation so that when a DNA segment is inserted into the site for insertion of a DNA segment, each promoter is operably linked to the DNA segment.

35. The plasmid of claim 34 further comprising:

a first enhancer operably linked to the first promoter; and

a second enhancer operably linked to the second promoter.

36. The plasmid of claim 34 or claim 35 further comprising:

a first transcriptional terminator, wherein the first transcriptional terminator is positioned so as to terminate transcription directed by the first promoter; and

a second transcriptional terminator, wherein the second transcriptional terminator is positioned so as to terminate transcription directed by the second promoter.

37. The plasmid of claim 36 further comprising a DNA segment inserted at the site for insertion of a DNA segment.

38. The plasmid of claim 37 wherein the sequence of the DNA segment comprises at least one sequence at least 80% identical to a portion of the sequence of at least one insect gene, wherein the portion of the sequence of the at least one insect gene is at least 50 nucleotides in length.

39. The plasmid of claim 37 wherein the sequence of the DNA segment comprises a plurality of sequences, wherein each sequence is at least 80% identical to a portion of the sequence of at least one insect gene, wherein the portion of the sequence of the at least one insect gene is at least 50 nucleotides in length.

40. The plasmid of claim 38 wherein the promoters are viral promoters.

41. The plasmid of claim 40 wherein the viral promoters are immediate early or early viral promoters.

42. The plasmid of claim 40 wherein the enhancers are viral enhancers.



43. The plasmid of claim 38 wherein the plasmid is a baculovirus transfer plasmid.

44. The plasmid of claim 43 wherein the promoters are selected from the group consisting of: the baculovirus *IE-1* promoter, the baculovirus *IE-2* promoter, the baculovirus *p35* promoter, the baculovirus *p10* promoter, and the baculovirus *polh* (polyhedrin) promoter.

45. The plasmid of claim 43 wherein the enhancers are selected from the group consisting of: the baculovirus *hr5*, *hr1*, *hr1a*, *hr2*, *hr3*, *hr4a*, *hr4b*, and *hr4c* enhancers.

46. The plasmid of claim 43, wherein the transcriptional terminator is selected from the group consisting of: the SV40 transcriptional terminator, the  $\beta$ -globin enhancer, and the baculovirus *polh* (polyhedrin) 3' untranslated region.

47. The plasmid of claim 38 wherein at least one of the genes is an essential gene in a pest organism.

48. The plasmid of claim 38 wherein at least one of the genes is involved in development in a pest organism.

49. The plasmid of claim 38 wherein at least one of the genes encodes a protein involved in neurotransmission in a pest organism.

50. The plasmid of claim 38 wherein at least one of the genes encodes a protein that is expressed in the insect alimentary canal or Malpighian tubules.

51. The plasmid of claim 38 wherein at least one of the genes is naturally found in an insect selected from the order *Lepidoptera*.

52. The plasmid of claim 38 wherein at least one of the genes is naturally found in an insect selected from the list consisting of: the cotton bollworm (*Helicoverpa zea*), the cabbage looper (*Trichoplusia ni*), the alfalfa looper (*Autographa californica*), the tobacco hornworm (*Manduca sexta*), the tobacco budworm (*Heliothis virescens*), the fall armyworm (*Spodoptera frugiperda*), the European corn borer (*Ostrinia nubilalis*), the eastern spruce budworm (*Choristoneura fumiferana*), the western spruce budworm (*C. occidentalis*), and the gypsy moth (*Lymantria dispar*).

53. The agent of claim 1 wherein the pest to be controlled is an insect.

54. The agent of claim 1 wherein the pest to be controlled is a nematode.

55. An insect containing the agent of claim 1.

56. An insect cell line containing the agent of claim 1.

57. An insect modified to transcribe at least one ribonucleic acid (RNA) that, when present within a cell, forms a double-stranded structure that inhibits expression of a target gene expressed in the cell.

58. A method of inhibiting expression of a gene within an insect cell comprising contacting the cell with a virus that directs transcription of at least one ribonucleic acid (RNA) that, when present within a cell, forms a double-stranded structure

59. The method of claim 58 wherein the virus is a baculovirus.

60. A method of controlling pests, the method comprising the step of:

5       introducing into one or more cells in a pest a molecule comprising dsRNA whose  
sequence corresponds to at least a portion of at least one gene endogenous to the  
pest, so that one or more biological or physiological functions of the pest is  
inhibited.

10       61. The method of claim 60, wherein the step of introducing comprises contacting the  
insect with a virus that expresses the dsRNA when introduced into cells.

15       62. The method of claim 61, wherein the virus expresses the dsRNA within 6 hours of  
infection.

20       63. The method of claim 61, wherein the virus expresses the dsRNA substantially in the  
absence of viral replication.

25       64. The method of claim 61, wherein the virus does not establish a productive infection.

65. The method of claim 60, wherein the gene portion is at least 50 nucleotides long.

66. The method of claim 60, wherein the gene portion is species specific in that it does  
not show more than approximately 80% homology with any known gene in any species  
other than the species in which the gene is naturally found.

67. The method of claim 60, wherein the gene portion is species specific in that it does not show more than approximately 80% homology with any known vertebrate gene.

68. The method of claim 61 wherein the step of contacting comprises applying the virus to organisms on which the pest feeds.

69. An insecticidal composition comprising the agent of claim 1 and an agriculturally suitable carrier.

70. The composition of claim 69 further comprising at least one agent selected from the group consisting of: conventional pesticides, gustatory stimulants, thickening agents, UV screening agents, optical brighteners, viral synergists, dispersants, flow agents, spreading agents, and sticking agents.

71. An insecticidal composition comprising the recombinant insect virus of claim 23 and an agriculturally suitable carrier.

72. The composition of claim 71 further comprising at least one agent selected from the group consisting of: conventional pesticides, gustatory stimulants, thickening agents, UV screening agents, optical brighteners, viral synergists, dispersants, flow agents, spreading agents, and sticking agents.

ADD add B9  
Al